STEROIDS CLIX

Antimicrobial Properties of 21,21-Dimethoxy Progesterone and Other Progesterone Analogues[1]

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Antimicrobial properties of steroid compounds have been recorded in the literature from time to time. Although Faulkner (1943) was not able to demonstrate definite antimicrobial effects of several natural estrogenic compounds, diethylstilbestrol did exhibit some degree of bactericidal action on gram-positive bacteria. Reiss (1947*a*, *b*) observed inhibitory effects of methyl testosterone and deoxycorticosterone on the growth of *Trichophyton purpureum* and *Trichophyton gypseum* in cultures and a certain curative effect on experimental infection in castrated rabbits. The antimycotic properties of androgenic and estrogenic compounds having the position 3 blocked with methyl groups, was pointed out by Rebell and Lamb (1953). Kull, Castellano, and Mayer (1953) reported on the inhibitory effect of water-soluble amino steroids on the growth of mycobacteria and Nocardiae. Fox, Carroll, and Glacy (1957) observed that none of the naturally occurring estrogens had fungistatic action; ethynyl estradiol showed some inhibitory effect on *Nocardia asteroides* at very high levels. Recently the antimicrobial properties of deoxycorticosterone on *Neurospora crassa* and other microorganisms have been pointed out (Lester, Stone, and Hechter, 1958; Kurosawa, 1958; Lester and Hechter, 1958, 1959; Chattaway, Townsley, and Barlow, 1959) and the inhibition of *Mycobacterium tuberculosis* by corticosterone and hydrocortisone was also indicated (Hennes et al., 1959). The inhibition of growth of *Tetrahymena piriformis* by several C-21 steroids was furthermore observed by Conner (1959).

During our studies on microbial transformations of steroids we have observed several effects of steroid compounds on growth, spore germination, germ tube growth, or activities of some microorganisms. On the basis of these cursory observations, a detailed investigation was undertaken to ascertain the effects of various progesterone analogues on the growth and activities of a selected group of bacteria, actinomycetes, and fungi, including animal and plant pathogens.

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MATERIALS AND METHODS

Organisms. The microorganisms used for these experiments were originally obtained from the American Type Culture Collection, from other institutions, or isolated from natural sources.

Inhibition of radial growth. Sabouraud-glucose-agar or potato-glucose-agar plates were prepared containing graded amounts of the steroid compounds (1 to 100 μg per ml); the compounds were incorporated into the melted agar in ethanol solution keeping the solvent concentration below the inhibitory levels. Discs of 4 mm in diameter of 1-week-old mycelial growth of the test fungus were used as inoculum. When testing dermatophytes, a standard blended mycelium obtained from submerged culture was utilized as inoculum in a procedure similar to that described by Hok et al. (1956). Depending on the organism, the plates were incubated to 28 or 37 C during periods of time ranging from 7 to 14 days. Measurements of the radial growth were recorded and the results given in terms of percentage of inhibition, taking the control culture as 100 per cent growth.

Inhibition of growth in shaken cultures. The test organisms were grown in liquid media containing graded amounts of the steroid (1 to 100 μg). In the case of fungi and actinomycetes, 25 ml of a medium containing 2 per cent peptone and 1 per cent glucose in distilled water dispensed in 125-ml Erlenmeyer flasks were used. After inoculation with 0.1 ml of spore (2 to 3 million per ml) or mycelial (5 mg dry weight per flask) suspensions, the cultures were incubated with agitation using rotary shakers. After 24 to 72 hr the mycelium was separated, dried to 80 C for

24 hr, and weighed. In a few instances growth was estimated as volume of wet cellular material.

Inhibition of spore germination. For this test, the slide method recommended by the American Phytopathological Society (1943) was used.

Minimal inhibitory concentration. The agar-streak dilution method (Waksman and Reilly, 1945) was used to determine the minimal inhibitory concentration of the steroids on bacteria, actinomycetes, and fungi, using in each case the appropriate agar medium: Sabouraud-glucose or potato-glucose-agar for testing fungi, nutrient agar in the case of bacteria, and Emerson's medium for actinomycetes. A dilution method using Proskauer and Beck's medium (Youmans, Doub, and Youmans, 1953; Bojalil and Medina, 1959) was used to test the inhibitory effects of the steroid on pathogenic mycobacteria and Nocardiae. Readings were recorded at the appropriate times, as a rule 24 hr for bacteria and 48 hr to 1 week for fungi; with the pathogenic mycobacteria the incubation period was extended for 4 weeks.

Metabolic studies. The inhibitory effect of 21,21-dimethoxy progesterone on the conversion of Reichstein's compound S to hydrocortisone by *Curvularia lunata* was studied in liquid shaken cultures. Peptone-glucose medium, 25 ml in 125-ml Erlenmeyer flasks, was sterilized and inoculated with spores or mycelial suspensions. In a set of cultures, amounts of steroid ranging from 1 to 100 μg per ml were added to the culture medium along with the inoculum and 10 mg of the steroid to be converted (compound S); in another set the organism was first grown for 48 hr and then graded concentrations of 21,21-dimethoxy progesterone and 10 mg of compound S were added. After 24 hr of oxidation, the cultures were extracted with methyl dichloride and the extracts analyzed by paper chromatography (Zaffaroni, 1953). The conversion product and the remaining starting material were determined by ultraviolet spectroscopy (240 mμ) after elution of the spots with methanol.

TABLE 1

Inhibition of radial growth of Curvularia lunata and Trichophyton mentagrophytes by progesterone analogues

Steroids (100 μg/ml)	Inhibition of Radial Growth	
	C. lunata	*T. mentagrophytes*
	%	%
21,21-Dimethoxy progesterone	85	68
21,21-Diethoxy progesterone	89	45
21,21-Diisopropyl progesterone	59	30
21,21-Dibenzyl progesterone	12	9
21-Methoxy progesterone	40	74
Δ^1-21,21-Dimethoxy progesterone	20	48
4-Pregnene-21-al-3,20-dione	14	50
Progesterone	17	72
19-Norprogesterone	34	84
Pregnane-21,21-dimethoxy-3,20-dione	14	65
Allopregnane-21,21-dimethoxy-3,20-dione	24	37
Allopregnane-21,21-dimethoxy-3-β-hydroxy-20-one	4	18
Allopregnane-21,21-dimethoxy-3-β-hydroxy-11,20-dione	9	62
4-Chloro-21,21-dimethoxy progesterone	18	61
11-α-Hydroxy progesterone	32	36
11-α-Hydroxy-19-norprogesterone	7	34
17-α-Hydroxy progesterone	7	24
17-Acetoxy progesterone	33	0
4-Chloro-progesterone	6	7
6-α-Fluoro-17-acetoxy progesterone	0	5
6-α-Methyl-17-α-hydroxy progesterone	0	4
6-β-Nitro progesterone	15	22
Pregnenolone	35	41
16-Dehydro progesterone	29	50
Deoxycorticosterone	25	80
Corticosterone	10	27
Reichstein's compound S	16	56
Hydrocortisone	0	5
Cortisone	0	11
17-α-Ethynyl testosterone	0	5

RESULTS AND DISCUSSION

From a group of 30 progesterone analogues tested for inhibitory action on radial growth of *C. lunata* and *Trichophyton mentagrophytes*, 21,21-dimethoxy progesterone (DMP) and 21,21-diethoxy progesterone showed the highest fungistatic effect, the first organism being the most sensitive (Table 1). The fungistatic action of dimethoxy progesterone decreased with the complexity of modifications at C-21, as shown in the case of diisopropyl and benzyl derivatives. It is interesting to note the reversal of the comparative sensitivity of *C. lunata* and the dermato-

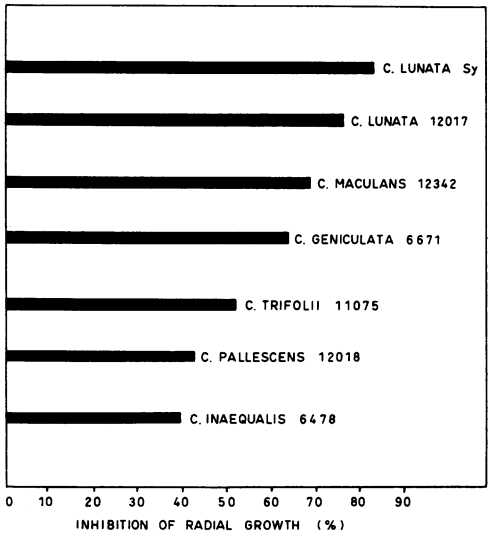

Fig. 1. Comparative effect of 21,21-dimethoxy progesterone on radial growth of several species of *Curvularia*.

phyte as influenced by modifications of the steroid structure. Modifications at C-21, such as presented by 21-methoxy progesterone or 4-pregnene-21-al-3,20-dione, modified the fungistatic effect on *C. lunata*, the inhibitory activity on the dermatophyte being affected to a lesser degree. The same activity pattern was shown by progesterone, 19-norprogesterone, deoxycorticosterone, and Reichstein's compound S, although further modifications such as 11-β-hydroxylation of compound S or deoxycorticosterone practically abolished the activity on *C. lunata*; compound S, however, retained significant activity on *T. mentagrophytes*. The elimination of the double bond between C-4 and C-5 in 21,21-dimethoxy progesterone giving the corresponding pregnane (5-β-hydrogen) or allopregnane (5-α-hydrogen) compounds (pregnane - 21,21 - dimethoxy - 3,20-dione and allopregnane-21,21-dimethoxy-3,20-dione) caused definite variation in the fungistatic activity; the 5-β-dihydro compound gave the same activity pattern as progesterone on *C. lunata* and *T. mentagrophytes* and the allo compound showed restricted fungistasis on both test organisms. Reduction of 21,21-dimethoxy-allopregnane-3,20-dione at C-3 did not modify the relative activity although the fungistatic effect was more limited; the introduction of oxygen at C-11 in the same compound enhanced the activity on the dermatophyte but not on

TABLE 2

Microorganisms which were not affected by 21,21-dimethoxy progesterone at concentration of 100 μg per ml

Bacteria	Yeasts
Escherichia coli RU (streptomycin resistant)	*Candida albicans* HI
Escherichia coli ATCC 9723	*Candida utilis* NRRL Y-900
Salmonella typhi HI	*Candida utilis* ATCC 9248
Salmonella typhimurium 528 HI	*Endomycopsis fibuliger* NRRL Y-1070
Shigella shiga HI	*Hansenula anomala* ATCC 8170
Shigella flexneri HI	*Rhodotorula gracilis* NRRL Y-1091
Proteus mirabilis HI	*Saccharomyces carlsbergensis* ATCC 9080
Klebsiella pneumoniae ATCC 10031	*Saccharomyces cerevisiae* ATCC 9763
Pseudomonas aeruginosa ATCC 10145	*Schizosaccharomyces pombe* ATCC 2476
Pseudomonas fluorescens ATCC 11251	*Torulopsis albida* NRRL Y-400
Pseudomonas oleovorans ATCC 8062	*Torulopsis minor* NRRL Y-1341
Pseudomonas testosteroni ATCC 11996	*Torulopsis sphaerica* NRRL Y-1101
Erwinia ananas E-B, ENCB	
Erwinia ananas E-C, ENCB	
Agrobacterium radiobacter ATCC 10311	
Rhizobium phaseoli ATCC 10321	
Rhizobium trifolii ATCC 10328	

Test organisms were originally received from the following institutions: RU, Rutgers University; ATCC, American Type Culture Collection; HI, Hospital Infantil, México, D. F. through Dr. J. Olarte; ENCB, Escuela Nacional de Ciencias Biólogicas, México, D. F.; NRRL, Northern Utilization Research and Development Division, U. S. Department of Agriculture.

C. lunata. The introduction of chlorine at C-4 decreased the activity of 21,21-dimethoxy progesterone in particular on *C. lunata* and the introduction of 11-α-hydroxy, 17-α-hydroxy, 6-β-nitro, or 6-α-methyl groups in the progesterone molecule had a similar effect, decreasing considerably the fungistatic properties of the steroid at least on one of the test organisms. The combined introduction of 17-α-hydroxy

TABLE 3

Inhibition of bacteria by 21,21-dimethoxy progesterone

Bacteria	Minimal Inhibitory Concn
	μg/ml
Agar-streak dilution method; 37 C:	
Bacillus subtilis ATCC 6633	50
Staphylococcus aureus ATCC 6538P	75
Mycobacterium phlei, RU	75
Mycobacterium rhodochrous ATCC 4273	37.5
Mycobacterium rhodochrous ATCC 4277	12.5
Mycobacterium rhodochrous ATCC 9356	25.0
Mycobacterium smegmatis ATCC 607	100
Mycobacterium smegmatis ATCC 599	50
Tube-dilution method; 37 C:	
Mycobacterium sp. UP, P-18 (photo-chromogen)	10
Mycobacterium sp. UP, P-50 (scoto-chromogen)	1
Mycobacterium smegmatis ATCC 607	100
Mycobacterium tuberculosis ATCC H37 Rv	50
Mycobacterium tuberculosis ATCC H37 Ra	100
Mycobacterium tuberculosis UP, INH-50 (isoniazid resistant)	25

Test organisms received from the institutions indicated in previous table. We are indebted to Dr. L. F. Bojalil from the Unidad de Patología, Facultad de Medicina, U. N. A. M., for tube dilution tests with a pathogenic Mycobacterium.

TABLE 4

Inhibition of actinomycetes by 21,21-dimethoxy progesterone

Actinomycetes	Minimal inhibitory Concn
	μg/ml
Agar-streak dilution method:	
Nocardia asteroides ATCC 9969	25
Nocardia asteroides UP, R-217	25
Nocardia asteroides UP, 60	10
Nocardia asteroides ATCC 8674	9
Nocardia convoluta ATCC 4275	50
Nocardia gardneri ATCC 9604	50
Streptomyces albus NRRL B-1333	100
Streptomyces coelicolor ENCB	100
Streptomyces lavendulae ATCC 8664	50
Streptomyces olivaceus ATCC 11624	100
Streptomyces roseochromogenes ATCC 3347	50
Tube dilution method:	
Nocardia asteroides UP, 60	5
Nocardia asteroides UP, 44	10
Nocardia brasiliensis UP, 41	10
Nocardia brasiliensis ISET, 416	5

Test organisms received from the institutions indicated in previous tables; ISET, Instituto de Salubridad y Enfermedades Tropicales, México, D. F. We are indebted to Dr. L. F. Bojalil from the Unidad de Patología for tube dilution tests with a pathogenic Nocardia.

and 6-α-methyl groups or 6-α-fluoro and 17-acetoxy groups in the progesterone molecule deprived the steroid of fungistatic properties on *C. lunata*. On the other hand, the fungistatic effect on *T. mentagrophytes* was influenced to a lesser degree by specific modifications of the dimethoxy progesterone molecule, but complexity of groups attached to C-21 and reduction of Δ^4-3-keto group, decreased the fungistatic action to some extent. Progesterone, 19-norprogesterone, and deoxycorticosterone were highly active on *T. mentagrophytes*, the fungistasis being affected by further modifications in the steroid molecule as hydroxylations in position 11 or 17, singly or in combination; this can be illustrated comparing the effect of 19-norprogesterone and the 11-α-hydroxy derivative or compound S and hydrocortisone. The introduction of chlorine or fluorine nullify the fungistatic activity exhibited by the parent steroid. In a recent study, Lester et al. (1958) demonstrated that the inhibitory effect of deoxycorticosterone on the growth of *N. crassa* was almost lost by esterification at C-21, unsaturation of ring A, reduction of Δ^4-3-ketone function, or introduction of hydroxyl groups. Conner (1959), studying the effect of steroids on the growth of *T. piriformis*, observed that the presence of the side chain was necessary for inhibitory action; the introduction of oxygen at C-21 or C-11 enhanced the effect, but hydroxylation at C-17, unsaturation of ring A, and substitution at C-9 lowered the inhibitory properties. It is clear from these observations that antimicrobial properties are not only dependent on the steroid structure but also on the particular test organism. In this respect, a number of species of *Curvularia* showed different degrees of sensitivity to dimethoxy progesterone (Fig. 1).

TABLE 5

Inhibition of pathogenic fungi by 21,21-dimethoxy progesterone

Fungi	Minimal Inhibitory Concn
	μg/ml
Colletotrichum coffeanum CF-2 ENCB	50–100
Colletotrichum gloesporioides CBS	100
Curvularia lunata 190 Sy	50
Fusarium moniliforme ATCC 9851	100
Fusarium solani CBS	100
Microsporum canis ATCC 9865	50
Rhizoctonia solani ATCC 10145	50
Trichophyton mentagrophytes ISET	25–50
Trichophyton mentagrophytes ATCC 9129	25–50
Trichophyton rubrum ISET	25–50

Test organisms received from the institutions indicated in previous tables; CBS, Centraalbureau voor Schimmelcultures; Sy, Syntex Collection.

In view of these results, a more extensive study was carried out to determine the antimicrobial spectrum of the compound using dilution methods; the highest amount of steroid utilized was 100 μg per ml. The results revealed that the steroid was inactive on gram-negative bacteria (Table 2); however, some gram-positive bacteria were inhibited at concentrations ranging from 1 to 100 μg per ml (Table 3). It was observed that mycobacteria were the most sensitive organisms, particularly photochromogens and scotochromogens. The photochromogen, strain P-18, is resistant to 50 μg per ml of isoniazid or streptomycin. A particular strain of *M. tuberculosis*, resistant to 50 μg per ml of isoniazid, showed sensitivity to 25 μg per ml of the steroid. These results are similar to those previously reported by Kull et al. (1953) who observed that *Mycobacterium smegmatis* strain 607 and *M. tuberculosis* strain H37Rv were inhibited in vitro by several amino steroids at concentrations between 30 and 130 μg per ml. Furthermore, Lester and Hechter (1958) indicated the inhibitory effect of deoxycorticosterone on *Mycobacterium ranae* at concentrations from 150 to 250 μg per ml and Hennes et al. (1959) found that hydrocortisone and cortisone inhibited the growth of *M. tuberculosis* at concentrations of 100 μg per ml, although 20 μg per ml gave slight inhibition. No differences were observed by these authors with isoniazid sensitive or resistant strains. 21, 21-Dimethoxy progesterone was active on *M. tuberculosis* strain H37Rv at 50 μg per ml, however, some differences in sensitivity can be observed with different isolates.

A number of species of *Nocardia*, both saprophytic and pathogenic, were inhibited by dimethoxy progesterone at concentrations from 5 to 50 μg per ml, *N. asteroides* and *Nocardia brasiliensis* being the most sensitive (Table 4). In this connection it is interesting to note that several workers indicated the inhibitory effect of some steroids on Nocardiae. Kull et al. (1953) reported the inhibition of growth of *N. asteroides* by 3-keto-21-(1-piperidyl)-4, 17-pregnadiene mono-hydrobromide at concentrations between 10 to 20 μg per ml, and Rebell and Lamb (1953) indicated a similar effect of 3-β-methoxy-17-β-hydroxy - Δ^5 - androstene - 17 - α - acetate at a concentration of 10 μg per ml. Data in Fig. 3 show the growth of *N. asteroides* strain 8694 in shaken cultures was inhibited by amounts of dimethoxy progesterone ranging from 1 to 25 μg per ml; apparently the inhibition rate was higher than observed in the case of *C. lunata* and *T. mentagrophytes*.

No activity was noticed against yeasts (Table 2) but several filamentous fungi were inhibited at concentrations between 25 and 100 μg per ml (Table 5). Particular attention was given to the study of the inhibitory effect of dimethoxy progesterone on *C. lunata*, an organism utilized to carry out the 11-β-hydroxylation of some steroid compounds (Shull, Kita, and Davisson, 1953). Both radial and mycelial growth in shaken cultures were used to ascertain the antifungal effect. Concentrations of dimethoxy progesterone between 6.25 and 100 μg per ml limited the radial growth significantly (Fig. 2). Concentrations of dimethoxy progesterone from 1 to 10 μg per ml were inhibitory for mycelial growth in shaken cultures (Fig. 3). As indicated in the results corresponding to 72 hr growth, the fungistatic effect on *C. lunata* was definitely transitory. In this regard, further experiments demonstrated that the fungus is able to metabolize the dimethoxy progesterone molecule converting it into more polar unidentified compounds having diminished fungistatic properties; when isolated and tested for inhibition of radial growth on *C. lunata*, these compounds showed 30 to 60 per cent less activity than the original one. The growth rate recovery of *C. lunata* runs parallel with the modification of the dimethoxy progesterone molecule. This behavior resembles

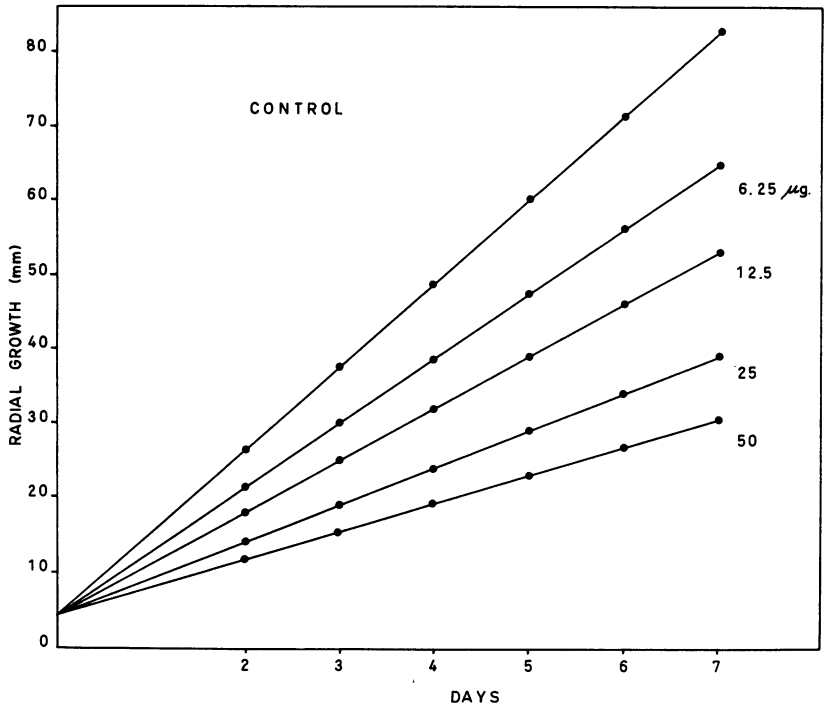

Fig. 2. Effect of various concentrations of 21,21-dimethoxy progesterone on radial growth of *Curvularia lunata.*

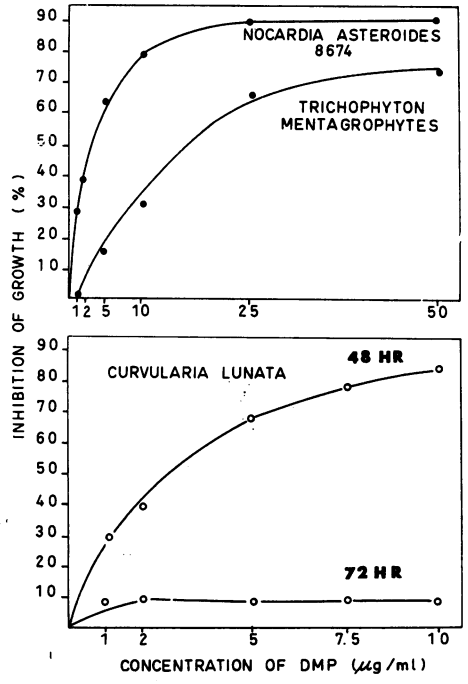

Fig. 3. Effect of 21,21-dimethoxy progesterone (*DMP*) on microbial growth in submerged culture. Growth of *Nocardia asteroides* was estimated as volume of wet cellular material.

that previously indicated in studies with *N. crassa* (Lester et al., 1958) and *Rhizopus nigricans* (Capek, Pavlu, and Hanc, 1958) postulating a detoxifying mechanism during the microbial transformation of steroids. Complementary experiments demonstrated definite fungistatic effects by using spores or mycelial inoculum. Spore germination was inhibited by concentrations of steroid as low as 2.5 μg per ml giving 25 per cent inhibition in 6 hr; 50 μg per ml gave 80 per cent inhibition under the same conditions (Fig. 4). The effect of steroid on *C. lunata* was compared with the activity on several plant and human pathogenic fungi (Fig. 5). Most of the inhibitory effect of dimethoxy progesterone on *C. lunata* and *Helminthosporium sativum* was attained at levels between 1 and 10 μg per ml and above this point the inhibition proceeded at a very low rate. In contrast, *T. mentagrophytes* was inhibited at a higher rate between 10 and 100 μg per ml. Moderate inhibition was obtained with the other test organisms.

It was observed that several steroids and precursors (cortisone, hydrocortisone, ergosterol, squalene) did not affect the growth of *C. lunata.* When tested in mixtures with dimethoxy progesterone, these compounds did not modify the

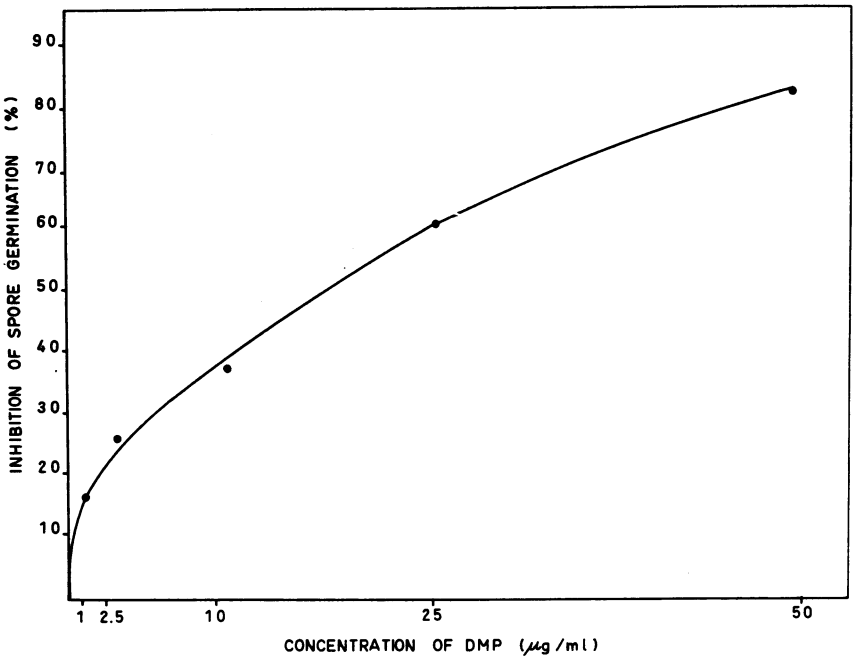

Fig. 4. Effect of 21,21-dimethoxy progesterone (*DMP*) on spore germination in *Curvularia lunata*

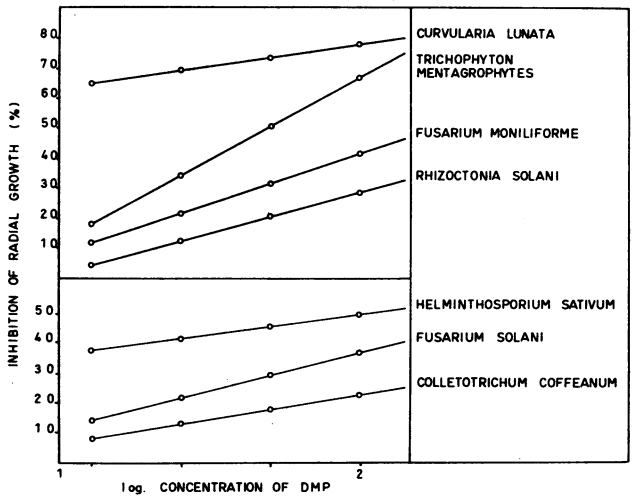

Fig. 5. Comparative effect of 21,21-dimethoxy progesterone (*DMP*) on pathogenic fungi

inhibition of radial growth (Table 6). In shaken cultures, however, a reversible action was observed with squalene and deoxycorticosterone at concentrations between 1 and 20 μg per ml. No clear explanation can be given for this behavior but experimental evidence obtained so far indicates that under submerged conditions the presence on either squalene or deoxycorticosterone increases the conversion rate of dimethoxy progesterone to less toxic compounds. Ergosterol was stimulatory and hydrocortisone did not modify the inhibitory effect (Table 7).

The results obtained in the study of the effect of dimethoxy progesterone on the course of the transformation of Reichstein's compound S to hydrocortisone by a particular strain of *C. lunata* are shown in Fig. 6. When dimethoxy progesterone was added to the culture medium

TABLE 6

Effect of 21,21-dimethoxy progesterone on radial growth of Curvularia lunata in the presence of other steroids

Steroids	Concn	Inhibition Range
	μg/ml	%
21,21-Dimethoxy progesterone (DMP)	100	75–85
Deoxycorticosterone	50	14–20
Cortisone	50	0–11
Hydrocortisone	50	0
17-β-Estradiol	50	33
Estrone	50	17–18
Ergosterol	50	0
Squalene	50	0
DMP + deoxycorticosterone	100 + 50	76–78
DMP + cortisone	100 + 50	75–78
DMP + hydrocortisone	100 + 50	73–77
DMP + estradiol	100 + 50	65–76
DMP + ergosterol	100 + 50	65–75
DMP + squalene	100 + 50	65–75

along with the inoculum and the steroid to be converted (compound S), a striking decrease in the 11-β-hydroxylating reaction was observed (curve A); concentrations ranging from 1 to 10 μg per ml inhibited the conversion to a considerable extent (30 to 86 per cent). Increased amounts of the inhibitory steroid gave the same effect; this behavior suggests that some limiting mechanisms, such as steroid solubility or binding on the mycelial surface, were in operation. When the compound was added after a 48-hr growing period along with the compound S, the inhibitory effect on the 11-β-hydroxylation was lessened, requiring higher concentrations of dimethoxy progesterone to attain the same degree of inhibition (curve B). This result indicates that dimethoxy progesterone interferes with the formation and activity of the 11-β-hydroxylating mechanisms in *C. lunata*. However, experiments utilizing a particular strain of *Cunninghamella bainieri*, which converts compound S into a mixture of epimeric compounds (hydrocortisone and epi-compound F), and with adrenal gland homogenates, which convert compound S into hydrocortisone, demonstrated that the steroid has no appreciable effect on the enzymatic action (Fig. 6). This indicates that a specific mechanism is acting in the case of *C. lunata*.

TABLE 7

Effect of 21,21-dimethoxy progesterone on submerged growth of Curvularia lunata in the presence of other steroids

Steroids	Concn	Dry Weight per Flask
	μg/ml	*mg*
No steroid		88.0
Ergosterol	1	102.7
	5	101.8
	10	99.4
	20	103.8
Squalene	1	98.0
	5	81.4
	10	83.9
	20	84.0
Deoxycorticosterone	1	92.7
	5	85.3
	10	90.6
	20	88.1
Hydrocortisone	1	95.1
	5	92.0
	10	89.8
	20	84.0
21,21-Dimethoxy progesterone (DMP)	20	54.0
Squalene + DMP	1 + 20	64.2
	5 + 20	56.0
	10 + 20	72.9
	20 + 20	75.9
Ergosterol + DMP	1 + 20	53.0
	5 + 20	72.6
	10 + 20	108.4
	20 + 20	111.8
Deoxycorticosterone + DMP	1 + 20	54.2
	5 + 20	74.0
	10 + 20	94.9
	20 + 20	92.4
Hydrocortisone + DMP	1 + 20	51.0
	5 + 20	53.3
	10 + 20	49.0
	20 + 20	53.4

The fungistatic action was parallel to the inhibitory effect on the conversion of compound S to hydrocortisone, which indicates interference with enzyme synthesis, although interference with cell permeability is not excluded. When the steroid was added to the system after 48 hr growth, at which point the 11-β-hydroxylating mechanisms were in operation, a definite inhibition of enzyme action was also observed.

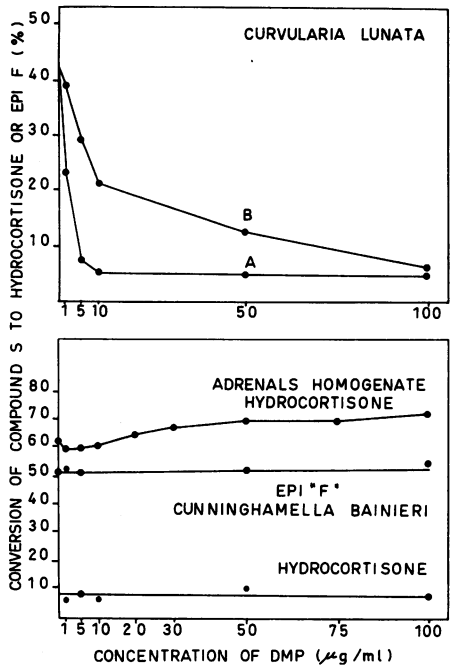

Fig. 6. Effect of 21,21-dimethoxy progesterone (*DMP*) on the conversion of Reichstein's compound S to hydrocortisone and epi- compound F.

SUMMARY

Thirty progesterone analogues were tested for inhibitory activity on radial growth of *Curvularia lunata* and *Trichophyton mentagrophytes*. 21, 21-Dimethoxy progesterone and 21, 21- diethoxy progesterone were the most active compounds on *C. lunata* and dimethoxy progesterone, progesterone, 19-norprogesterone, and deoxycorticosterone were most inhibitory on *T. mentagrophytes*. Dimethoxy progesterone also inhibited *Colletotrichum coffeanum*, *Fusarium moniliforme*, *Fusarium sativum*, and *Rhizoctonia solani*. Gram-positive bacteria, particularly saprophytic and pathogenic mycobacteria were inhibited by concentrations of dimethoxy progesterone ranging from 1 to 100 μg per ml. *Nocardia asteroides* and *Nocardia brasiliensis* showed sensitivity to the steroid at concentrations from 5 to 50 μg per ml. No inhibitory effect was observed on gram-negative bacteria and yeasts. Dimethoxy progesterone was essentially bacteriostatic or fungistatic and its effect on radial growth of *C. lunata* was not modified by inactive steroids (cortisone, hydrocortisone) or precursors (squalene); however, the inhibition of mycelial synthesis in submerged cultures was reversed by squalene or deoxycorticosterone. Under the same growth conditions, ergosterol was stimulatory and hydrocortisone inactive. The complexity of groups attached to C-21, the suppression of the double bond between C-4 and C-5, reduction of Δ^4-3 keto function, introduction of a double bond at C-1, and halogenation were modifications which brought about marked decrease in the fungistatic action of dimethoxy progesterone on *C. lunata*. The inhibition of *T. mentagrophytes* was influenced to a lesser degree by modifications of the steroid molecule. Further modifications of progesterone molecule resulted in an almost complete loss of activity on both test organisms. Metabolic studies in connection with the 11-β-hydroxylation of Reichstein's compound S by *C. lunata* demonstrated that dimethoxy progesterone at the concentrations from 1 to 10 μg per ml significantly inhibited the formation of hydrocortisone. Results indicate that the inhibitory steroid prevents both cellular synthesis and enzyme action in *C. lunata*. In contrast, dimethoxy progesterone did not affect the steroid hydroxylating abilities of *Cunninghamella bainieri* or adrenal gland homogenates.

REFERENCES

American Phytopathological Society 1943 Committee on standardization of fungicidal tests. The slide-germination method of evaluating protectant fungicides. Phytopathology, **33**, 627–632.

Bojalil, L. F., and B. Medina 1959 Acción de diversos agentes quimioterapeuticos sobre el crecimiento de algunas especies de *Nocardia*. Rev. latinoamer. microbiol., **2**, 33–40.

Capek, A., H. Pavlu, and O. Hanc 1958 Microbial transformation of steroids. XI. The influence of steroids on respiration. Česko-slov. mikrobiol., **3**, 154–158.

Chattaway, F. W., J. D. Townsley, and A. E. J. Barlow 1959 Effect of steroids and related compounds on the growth of dermatophytes. Nature, **184**, 1731–1732.

Conner, R. L. 1959 Inhibition of growth of *Tetrahymena piriformis* by certain steroids. J. Gen. Microbiol., **21**, 180–185.

Faulkner, G. H. 1943 Bactericidal action of estrogens. Lancet, **2**, 38–40.

Fox, L. E., W. R. Carroll, and J. Glacy 1957 Fungistatic action of estrogens and related compounds. J. Am. Pharm. Assoc., **46**, 276–278.

HENNES, A. R., H. G. MUCHMORE, H. G. MCCLURE, AND J. F. HAMMARSTEN 1959 In vitro inhibition of growth of *M. tuberculosis* by certain 11-oxygenated steroids. Proc. Soc. Exptl. Biol. Med., **101**, 145–147.

HOK, K. A., A. Y. HAMILTON, K. S. PILCHER, AND R. NIEMAN 1956 Antifungal activity of a new group of salicylamide derivatives for dermatophytes. Antibiotics & Chemotherapy, **6**, 456–464.

KULL, F. C., G. A. CASTELLANO, AND R. L. MAYER 1953 The in vitro antimicrobial activities of certain amino steroids. J. Invest. Dermatol., **21**, 227–228.

KUROSAWA, Y. 1958 Steroids and microbes. IV. Antibiotic action of steroids. Nippon Nôgei-kagaku Kaishi, **32**, 415–419.

LESTER, G., D. STONE, AND O. HECHTER 1958 The effects of deoxycorticosterone and other steroids on *Neurospora crassa*. Arch. Biochem. Biophys., **75**, 196–214.

LESTER, G., AND O. HECHTER 1958 Effect of deoxycorticosterone on the growth of microorganisms. J. Bacteriol., **76**, 365–367.

LESTER, G., AND O. HECHTER 1959 Regulation of the growth of *Penicillium puberulum* by estradiol and deoxycorticosterone. Bacteriol. Proc., **1959**, 114.

REBELL, G., AND J. H. LAMB 1953 In vitro study of a group of blocked steroids as antimycotic agents. J. Invest. Dermatol., **21**, 331–335.

REISS, F. 1947*a* The effect of hormones on the growth of *Trichophyton purpureum* and *Trichophyton gypseum*. J. Invest. Dermatol., **8**, 245–250.

REISS, F. 1947*b* The effect of sex hormones on experimental *Trichophyton purpureum* infection in rabbits. J. Invest. Dermatol., **8**, 251–253.

SHULL, G. M., D. A. KITA, AND J. W. DAVISSON 1953 Oxygenation of steroids. U. S. Patent 2,658,023.

WAKSMAN, S. A., AND C. H. REILLY 1945 Agar-streak method for assaying antibiotic substances. Ind. Eng. Chem., Anal. Ed., **17**, 556–558.

YOUMANS, G. P., L. DOUB, AND A. S. YOUMANS 1953 The bacteriostatic activity of 3500 organic compounds for *Mycobacterium tuberculosis* var. *hominis*. Chem. Biol. Center, N. R. C., Washington, D. C.

ZAFFARONI, A. 1953 Micromethods for the analysis of adrenocortical steroids. Recent Progr. in Hormone Research, **8**, 51–86.